

CAPACITY FOR BIOSYNTHESIS OF PROSTAGLANDIN-RELATED  
COMPOUNDS: DISTRIBUTION AND PROPERTIES OF THE  
RATE-LIMITING ENZYME IN HYDROCORALS, GOR-  
GONIANS, AND OTHER COELENTERATES OF  
THE CARIBBEAN AND PACIFIC

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The search for new marine sources of physiologically potent chemicals of interest to biology, and potential utility to medicine, agriculture, industry, and research, has in many cases been hindered by the lack of analytical procedures of sufficient generality, rapidity, and adaptability to use in the field. This has been particularly so in the case of the hormone-like prostaglandin-related compounds (PGRCs), which are now known to include a large and confusing multiplicity of prostaglandins (PGs), prostacyclins, thromboxanes, and prostaglandin-endoperoxides (Karim and Rao, 1975; Hamberg, Svensson and Samuelsson, 1975, Pace-Asciak and Wolfe, 1971; Johnson, Morton, Kinner, Gorman, McGuire and Sun, 1976).

Very high levels of the prostaglandins PGE<sub>2</sub>, PGA<sub>2</sub>, and certain of their related isomers have been found in different clonal populations of the Caribbean gorgonian, *Plexaura homomalla* (*Anthosoa: Gorgonacea*) (Weinheimer and Spraggins, 1969; Weinheimer, 1974; Light and Samuelsson, 1972; Schneider, Hamilton and Rhuland, 1972). This finding generated considerable interest in the potentials for development and conservation of this gorgonian as a major medical resource (Bayer and Weinheimer, 1974), although commercial interest in this fragile and slowly growing species (Kinzie, 1974; Hinman, Anderson and Simon, 1974; Jordan, Castanares and Ibarra, 1978) has been supplanted by recent improvements in synthetic methods for the production of some of the prostaglandins. The full extent of the distribution and potential resource of PGRCs from the marine invertebrates, as well as the functions of the PGRCs in these animals, remain largely unknown, however. There are well over a hundred prostaglandins and other PGRCs now recognized, with newly identified members of this family being discovered at an exponential rate (Karim and Rao, 1975). Assays are further complicated by the fact that these compounds are for the most part highly unstable (under physiological, aqueous, and aerobic conditions), and possess overlapping spectra of physical and biological properties, thus necessitating resolution and analysis by complicated and specialized techniques (Schneider, 1976; Salmon and Karim, 1976).

All of the PGRCs, however, are synthesized from a common (and unstable) intermediate: a prostaglandin-endoperoxide (PGE<sub>P</sub>) (Hamberg and Samuelsson, 1973, 1974). The enzyme-complex catalyzing the rate-limiting step in the biosynthesis of this central intermediate is known as prostaglandin-endoperoxide syn-

thetase (also known as prostaglandin synthetase or fatty acid cyclo-oxygenase) (Miyamoto, Ogino, Yamamoto and Hayaishi, 1976). Although several techniques for the assay of this enzyme are available (Samuelsson, Granström, Green, Hamberg and Hammarström, 1975; Salmon and Karim, 1976), use of these assays for the direct measurement of a tissue's maximal capacity for PGEP synthesis (and thus, the total capacity for subsequent biosynthesis of PGRCs) has been complicated by the pronounced autocatalytic and autodestructive activities of the enzyme during such procedures (Miyamoto *et al.*, 1976; Lands and Rome, 1976). Such marked deviations from simple first-order kinetics result in a complex, nonlinear proportionality of the reaction with respect to both time and the amount of enzyme present, thus limiting the usefulness of these techniques for comparative assessments of relative PGRC biosynthetic capacities.

The PGEP synthetase-catalyzed reaction is markedly stimulated by hydrogen peroxide ( $H_2O_2$ ), and the enzyme from many invertebrate sources appears to generate this activator autocatalytically during the course of its normal reaction (Morse, Duncan, Hooker and Morse, 1977, 1978). Addition of exogenous hydrogen peroxide (or addition of a hydrogen peroxide *generating system*) rapidly activates PGEP synthetase; the rate of the reaction catalyzed by this enzyme is then easily measured, and is directly proportional to the amount of the enzyme present. Based upon this finding, a rapid and convenient spectrophotometric micro-assay for PGEP synthetase (Takeguchi and Sih, 1972) was modified, especially adapting it for use in the field by inclusion of a stable enzymatic  $H_2O_2$ -generating system. Using this technique to measure the levels of PGEP synthetase in a variety of marine coelenterates from the Caribbean and Pacific, especially high specific activities of this enzyme were found in several of the plexaurid *Gorgonacea* (including *P. homomalla*), in three species of "*Hydrocorallia*" (*Milleporina* and *Stylasterina*), and in two species of *Hyroida*; significant levels of the enzyme were also found in species belonging to other orders, as well.

#### MATERIALS AND METHODS

##### *Specimen collection*

Marine coelenterates were obtained from both the Caribbean (Bonaire, Netherlands Antilles; July–August, 1976; 1–30 m depth) and the Pacific (Santa Barbara Channel, California; August, 1976–July, 1977; 0–15 m depth). Small samples of tissue (generally < 5 g, including associated skeletal and substrate material) were collected from these marine species and sealed, *in situ*, in separate polyethylene bags of sea water (*ca.* 100–200 ml); these were brought ashore for prompt assay. Freshwater hydroids were obtained from the Carolina Biological Corporation. Only fresh, live specimens were used for all assays reported here. Identification and classification of species were made according to Hyman (1940), Bayer (1961), Roos (1971), Boschma (1956), Smith (1971), Smith and Carlton (1975), Durham and Bernard (1952), Johnson and Snook (1955), and Allen (1976).

##### *Preparation of extracts*

All samples of marine species were rinsed with sea water after separation from associated substrate and other biota as necessary. Tissue was removed from

the samples of *Scleractinia*, *Milleporina* and *Stylasterina* by scraping the skeletal material with a scalpel, and irrigating with a small volume of chilled *tris*-hydroxymethylaminomethane (Tris)-HCl buffer (10 mM, pH 7.1, 0° C); all other samples were minced (at 0° C) to facilitate homogenization.

Small samples of weighed tissue (0.1–0.5 g) were homogenized (0° C) in 1–3 volume-equivalents of the above Tris-Cl buffer, using a small glass or Teflon Dounce homogenizer. Particulate material and debris were removed by brief low-speed centrifugation, and the extracts held at 0° C for immediate assay.

### Assays

The catalytic activity of PGEP synthetase was measured using a modification (Morse *et al.*, 1977) of the technique originally developed by Takeguchi and Sib (1972). This assay spectrophotometrically monitors the obligatory co-oxidation of the colorless aromatic cofactor, *L*-epinephrine, as it is converted to the intensely red adrenochrome product. The assay-mixture (1 ml, 20–23° C) contained Tris-Cl buffer (10 mM, pH 7.1), arachidonic acid as substrate and *L*-epinephrine as cofactor (each at 1 mM), with the extract to be assayed and other additions as indicated in the text. The course of the reaction was monitored as the rate of change in optical absorbance at 480 nm. For use in the field, assays were performed with a Bausch and Lomb Mini-Spectrophotometer (weight *ca.* 200 g) and stopwatch; assays performed in the laboratory made use of a Gilford recording spectrophotometer. Assays of the same homogenates performed in parallel with these two instruments were found to agree within  $\pm 9\%$ .

Where indicated (Table I), extracts were heated at 90° C for 10 min prior to assay, to denature enzyme protein. Also as indicated, catalase was added at 0.1  $\mu$ g/ml; phenylecyclopropylamine, a pirin, indomethacin, acetaminophen, DDTC, and EDTA were added at 1 mM concentration as shown.

Aliquots of extracts were stored frozen, and subsequently assayed for protein concentration by the method of Lowry, Rosebrough, Farr and Randall (1951). Specific activities are expressed as the change in absorbance (at 480 nm) in the assay mixture per minute per mg of added protein.

### Chemicals

Tris and Tris-Cl (pre-equilibrated to yield pH 7.1 at 10 mM, 22° C, *L*-epinephrine, glucose oxidase and catalase were obtained from the Sigma Chemical Co.; H<sub>2</sub>O<sub>2</sub> (30%, stabilized) was obtained from Mallinckrodt, and diluted just before use. Diethyldithiocarbamate (DDTC, sodium salt) and ethylenediaminetetraacetic acid (EDTA, tetrasodium salt) were from Fisher Chemical Corporation; all other chemicals were reagent grade. All solutions were prepared with distilled water.

## RESULTS

That the "prostaglandin A<sub>2</sub> synthetase complex" of *P. homomalla* is activated by 1 M NaCl (Corey, Washburn and Chen, 1973) was verified in this study, using the spectrophotometric assay for the PGEP synthetase reaction; this activation was found to be a general (although somewhat variable) property of the

TABLE I

*Properties of PGEP synthetase in extracts of Allopora porphyra. PGEP synthetase activity was assayed in 5  $\mu$ l aliquots of a freshly prepared extract (8.8 mg protein/ml) of Allopora porphyra as described in the text, with alterations as specified. Both the maximal rate of the enzyme-catalyzed reaction (in the presence of 1 M NaCl) and the initial rate (in the presence of 0.6 mM  $H_2O_2$ ) were measured; results are the averages ( $\pm$ s.d.) of duplicate determinations normalized to values obtained with the respective complete assay mixtures. The maximal rate of the reaction (+NaCl, measured after ca. 5 min) corresponds to 0.27  $\mu$ mole epinephrine oxidized per minute; the initial rate in the complete system with  $H_2O_2$  was 0.24  $\mu$ mole/min.*

Assay mixture	Relative activity (%)	
	Maximal rate (with NaCl)	Initial rate (with $H_2O_2$ )
Complete system	100 $\pm$ 8	100 $\pm$ 6
Omit activator (NaCl or $H_2O_2$ )	41 $\pm$ 5	2 $\pm$ 1
+ catalase	0 $\pm$ 0	0 $\pm$ 0
+2x Extract	198 $\pm$ 4	208 $\pm$ 9
Omit extract	0 $\pm$ 0	0 $\pm$ 0
+Heated extract	0 $\pm$ 0	0 $\pm$ 0
Omit arachidonic acid	38 $\pm$ 3	54 $\pm$ 2
+Phenylcyclopropylamine	3 $\pm$ 0	0 $\pm$ 0
+Aspirin	84 $\pm$ 6	54 $\pm$ 6
+Indomethacin	44 $\pm$ 5	16 $\pm$ 2
+Acetaminophen	61 $\pm$ 2	62 $\pm$ 7
+DDTC	0 $\pm$ 0	4 $\pm$ 1
+EDTA	133 $\pm$ 5	140 $\pm$ 12

enzyme from most of the coelenterates assayed. No such salt-stimulation of PGEP synthetases was observed in active extracts from marine echinoderms, molluscs, or fishes, however.

As the spectrophotometric assay affords a means for continuously monitoring the progress of the enzymatic reaction, the effect of salt upon the coelenterate PGEP synthetase could be studied in more detail. Addition of NaCl increases both the maximal (autocatalytic) rate and the final yield of the reaction by ca. 2-3 fold; there is no significant effect of salt on the slow *initial* rate, however. Final yield of the reaction is limited, in part, by an enzymatic, autoinhibitory process, and not by depletion of substrate. [Similarly complex autocatalytic and autoinhibitory processes also have been observed in kinetic analyses of the reaction catalyzed by PGEP synthetases from a variety of mammalian sources (*e.g.*, Lands and Rome, 1976; Miyamoto *et al.*, 1976).]

The data in Table I illustrate the properties of the PGEP synthetase in an extract of the Pacific "hydrocoral," *Allopora porphyra* (Hydrozoa: Stylasterina), a species especially rich in this enzyme. Similar properties were found for the PGEP synthetases in extracts of *P. homomalla* (Anthozoa: Gorgonacea), *Millepora* spp. (Hydrozoa: Milleporina), and *Sertularia turgida* and *Hydractinia milleri* (both Hydrozoa: Hydrozoa); thus, the data in Table I are generally representative of the PGEP synthetases from those coelenterates which contain significant quantities of this enzyme (*cf.* Table III).

As seen in Table I, the maximal rate of the autocatalytic reaction is stimulated ca. 2.5-fold by 1 M NaCl. By continuously recording the change in absorbance

during the spectrophotometric assay, this maximal rate of the salt-stimulated enzyme-catalyzed reaction can be determined with a high degree of accuracy, and is proportional to the amount of extract added. This same maximal rate ( $\pm 15\%$ ) can be obtained—with no autocatalytic lag—by providing hydrogen peroxide as activator in place of NaCl in the complete assay mixture. Maximal stimulation of the enzyme from coelenterate tissues was found to occur at approximately 0.6 mM  $H_2O_2$ ; this is close to the value of 0.3 mM previously found to give optimal stimulation of PGEP synthetase from eggs of the abalone, *Haliotis rufescens* (Morse *et al.*, 1977, 1978). As expected, the peroxide-stimulated reaction is completely inhibited by the addition of purified catalase, an enzyme which rapidly and specifically decomposes the added  $H_2O_2$  to water and oxygen. More significant, however, is the observation that both the autocatalytic activation, and all catalytic activity, seen in the absence of exogenous peroxide ( $\pm$  NaCl) are completely inhibited by a small concentration of catalase. This observation, also made with the PGEP synthetase from other marine invertebrates (Morse *et al.*, 1977, 1978), indicates that both the activity and autocatalytic activation of the enzyme from these sources normally depend upon the (autocatalytic) generation of  $H_2O_2$  by the PGEP synthetase itself.

Both the maximal rate of the salt-stimulated reaction and the initial (= maximal) rate of the peroxide-stimulated reaction are absolutely dependent upon a heat-labile factor (presumably enzyme) in the added extract (Table I). Dependence upon the added substrate, arachidonic acid, is only partial and widely variable from extract to extract, presumably reflecting the variable presence of endogenous lipid substrates in the crude extracts. Enzymatic activity in the presence of either NaCl or  $H_2O_2$  is inhibited to various extents by the pharmacological anti-inflammatory, analgesic and/or antipyretic agents phenylcyclopropylamine, aspirin, indomethacin, and acetaminophen; these agents are known to inhibit PGEP synthetases from a variety of different organisms and tissues with widely varying efficiencies (Lands and Rome, 1976). Phenylcyclopropylamine is most efficient, of these, at inhibiting the coelenterate enzyme; it had been observed previously that the salt-activated enzyme from *P. homomalla* was relatively insensitive to indomethacin, although lower concentrations of that agent than used in the present study had been employed (Corey *et al.*, 1973). Our data indicate, however, that the initial rate of the reaction catalyzed by the peroxide-activated coelenterate enzyme is significantly more sensitive to inhibition by aspirin and indomethacin than is the maximal rate achieved after autocatalytic activation in the presence of salt. As with the PGEP synthetases from other sources (Morse *et al.*, 1977; Letellier, Smith and Lands, 1973), the coelenterate enzyme is strongly inhibited by diethyl-dithiocarbamate (DDTC), a chelator strongly specific for copper. The addition of EDTA, a chelator which is specific for heavy metals other than copper, results in a slight but significant increase in catalytic activity. These latter observations suggest that copper may play some essential role in the coelenterate PGEP synthetase, as it does in many other oxygenases (Morse *et al.*, 1978); traces of other heavy metals appear to cause slight inhibition, which may be relieved by addition of EDTA.

The peroxide-stimulated reaction was further adapted for use in an assay which could be performed conveniently under field conditions, by replacement of

the  $H_2O_2$  with a stable, enzymatic  $H_2O_2$ -generating system. As seen in Table II, a simple enzymatic system (consisting of glucose oxidase and its substrate, *D*-glucose) can be incorporated in the spectrophotometric assay for the continuous production of  $H_2O_2$  (and glucuronic acid) *in situ*. The purified and concentrated glucose oxidase, which is inexpensively available from several commercial sources, proves to be fairly stable; such preparations can tolerate several weeks in transit without refrigeration, with little significant loss in activity. Using the "coupled assay" shown in Table II, with optimal concentrations of glucose and glucose oxidase replacing the direct addition of  $H_2O_2$ , the measured activity was found to exhibit dependence upon added coelenterate extract and substrate, and sensitivity to inhibitors, closely parallel to results obtained with the simple  $H_2O_2$ -stimulated reaction shown in Table I.

Using this convenient and readily portable assay procedure, the relative PGEP synthetase levels were measured in extracts of fresh, live tissue from a variety of coelenterates of the Caribbean and eastern Pacific (Table III). In addition to the very high levels of this enzyme found in *P. homomalla*, high or significant specific activities were found in four other species of plexaurids and *Gorgonia ventalina* (all Gorgonacea), the solitary Scleractinian, *Coenocyathus bowersi*, the Caribbean Antipatharian ("black coral") *Antipathes atlantica*, the Hydroids *Hydractinia milleri* and *Sertularia turgida*, and four species of *Milleporina* and *Stylasterina* (the "Hydrocorallia"). Enzymatic activities from all of these sources were found to be dependent upon  $H_2O_2$ . The low specific activities measured in the other species assayed actually reflect lower concentrations of the enzyme, rather than the presence of some inhibitor of its activity, as no significant inhibition was detected upon mixing any of the extracts (of all species tested) with extracts of *Plexaura*, *Millepora*, or *Allopora*.

The distribution of the enzyme in the plexaurid Gorgonacea and in the "Hydrocorallia" (*Milleporina* and *Stylasterina*) appears to be of some general significance, although few other taxonomic or physiological correlates of this distribution are apparent. It should be noted that if *Muricea* is included in the Plexauridae, as according to Bayer (1961), high levels of PGEP synthetase may not be entirely characteristic of this family; however, others have classified this genus

TABLE II

"Coupled assay" with endogenous generation of  $H_2O_2$  by glucose oxidase. PGEP synthetase activity was assayed in an extract of *Allopora porphyra* as described in Table I, except that the otherwise complete system contained "activator" as specified. Results are the averages ( $\pm$ s.d.) of duplicate determinations, normalized to the value obtained in the presence of  $H_2O_2$  at optimal concentration.

Activator	Initial Rate (%)
$H_2O_2$ (0.6 mM)	100 $\pm$ 1
None	2 $\pm$ 1
Glucose (5 mM)	2 $\pm$ 1
Glucose Oxidase (10 $\mu$ g)	14 $\pm$ 6
Gluc. (5 mM) + Gluc. Ox. (10 $\mu$ g)	115 $\pm$ 3
Gluc. (50 mM) + Gluc. Ox. (10 $\mu$ g)	82 $\pm$ 7
Gluc. (5 mM) + Gluc. Ox. (100 $\mu$ g)	83 $\pm$ 6

TABLE III

*Species-distribution of PGEP synthetase in coelenterates. Specimens were collected and assayed as indicated; specific activities are the averages ( $\pm$ s.d.) of results from two or more separate colonies measured in the "coupled" assay with endogenous generation of  $H_2O_2$  (5 mM glucose + 10  $\mu$ g/ml glucose; cf. Table II). Collection sites are: P, Pacific; C, Caribbean; f.w., freshwater. An asterisk denotes species with high specific activity of PGEP synthetase. In all cases in which significant activity ( $\geq 0.1$ ) was detected, proportionality of activity with added extract, and dependence upon  $H_2O_2$  were determined (as in Tables I and II). Absence of inhibitors in all extracts was verified as described in the text.*

Family	Species	Collection Site	PGEP Synthetase (Specific Activity)
(Anthozoa: Octocorallia)			
Plexauridae	<i>Plexaura homomalla</i> (var. <i>homomalla</i> )	C	* 9.0 $\pm$ 1.8
	(var. <i>kükenthali</i> )	C	* 9.0 $\pm$ 2.2
Plexauridae	<i>Plexaura flexuosa</i>	C	* 8.5 $\pm$ 1.6
Plexauridae	<i>Pseudoplexaura flagellosa</i>	C	* 5.8 $\pm$ 1.5
Plexauridae	<i>Eunicca tourneforti</i> (var. <i>tourneforti</i> )	C	* 3.0 $\pm$ 1.2
	(var. <i>atra</i> )	C	* 3.2 $\pm$ 0.3
Plexauridae	<i>Plexaurella dichotema</i>	C	* 2.0 $\pm$ 0.6
Gorgoniidae	<i>Gorgonia ventalina</i>	C	* 1.6 $\pm$ 0.3
Gorgoniidae	<i>Pseudopterogorgia americana</i>	C	$\leq 0.1 \pm 0$
Gorgoniidae	<i>Eugorgia rubens</i>	P	0.3 $\pm$ 0.1
Gorgoniidae	<i>Lophogorgia chilensis</i>	P	$\leq 0.1 \pm 0$
Gorgoniidae	<i>Filigella mitsukurii</i>	P	$\leq 0.1 \pm 0$
Muriceidae	<i>Muricea californica</i>	P	$\leq 0.1 \pm 0$
Clavulariidae	<i>Clavularia</i> sp.	P	0.3 $\pm$ 0.1
Virgulariidae	<i>Stylatula elongata</i>	P	$\leq 0.1 \pm 0$
Virgulariidae	<i>Acanthoptilum gracile</i>	P	0.2 $\pm$ 0.1
Renillidae	<i>Renilla köllikeri</i>	P	$\leq 0.1 \pm 0$
(Anthozoa: Hexacorallia)			
Seriatoporidae	<i>Mudracis decactis</i>	C	0.2 $\pm$ 0.1

as belonging to a separate group (as indicated in Table III). No significant differences were observed between two subspecies each of *P. homomalla* (var. *homomalla* and var. *kükenthali*; Table III), *Eunicca tourneforti* (var. *tourneforti* and var. *atra*; Table III), or *Allopora porphyra* (vars. red vs. orange; cf. Ostarello, 1973), when these pairs were collected and assayed in parallel. Similarly, no significant differences were observed (in parallel collections and assays) between PGEP synthetase levels in male and female colonies of dioecious species such as *Plexaura*, *Millepora*, or *Allopora*.

Corey and Washburn (1974) had previously shown that the PG synthetase complex of *P. homomalla* resides in the tissue of the animal, rather than in its symbiotic zooxanthellae. In view of the suggestions made by them and others (Corey and Washburn, 1974; Gonzalez, 1978) that photosynthetic products of the zooxanthellae may nevertheless contribute to (or control) the biosynthesis of PGRCs in coelenterates, it was of interest to determine the relative activities of PGEP synthetase from colonies of the same species exposed to widely differing regimes of illumination. However, we have found that colonies of *Millepora alcicornis* collected from depths of 1 m and 30 m (the extremes of its depth-

distribution which we observed), when assayed in parallel, showed no significant differences in specific activity, thus suggesting that photosynthetic activity may have little direct influence over the synthesis or activity of the rate-limiting enzyme, PGEP synthetase.

## DISCUSSION

In their studies of the PG synthetase from *P. homomalla*, Corey *et al.* (1973) found apparently complete dependence of activity upon added NaCl, whereas our assays detect only a 2–3 fold stimulation in extracts of this and other coelenterates. Possible reasons for the difference between these observations include the fact that Corey *et al.* measured the final yield of the overall enzymatic synthesis of PGA<sub>2</sub>, whereas we have measured the rate of the reaction catalyzed by PGEP synthetase alone. Also, Corey *et al.* measured the final cumulative activity in extracts which had been stored frozen, whereas our assays were performed with specimens which had been freshly collected and live immediately prior to assay. In fact, the activity of the PGEP synthetase complex was found in this study to be only partially stable in frozen tissues, with samples variably losing 50–80% of their activity when kept at  $-30^{\circ}\text{C}$  for two months.

TABLE III—Continued

Family	Species	Collection Site	PGEP Synthetase (Specific Activity)
Acroporidae	<i>Acropora palmata</i>	C	$\leq 0.1 \pm 0$
Agariciidae	<i>Agaricia agariciles</i>	C	$0.2 \pm 0$
Agariciidae	<i>Agaricia fragilis</i>	C	$0.2 \pm 0.1$
Faviidae	<i>Diploria labyrinthiformis</i>	C	$\leq 0.1 \pm 0$
Trochosmiliidae	<i>Meandrina meandrites</i>	C	$\leq 0.1 \pm 0$
Trochosmiliidae	<i>Dendrogyra cylindrus</i>	C	$0.3 \pm 0.1$
Eupsammidae	<i>Ballanophyllia elegans</i>	P	$0.3 \pm 0.1$
Astrangidae	<i>Astrangia lajollaensis</i>	P	$0.5 \pm 0.2$
Caryophylliidae	<i>Coenocyathus bowersi</i>	P	* $3.3 \pm 0.4$
Anthopleuridae	<i>Anthopleura elegantissima</i>	P	$\leq 0.1 \pm 0$
Anthopleuridae	<i>Anthopleura xanthogrammica</i>	P	$\leq 0.1 \pm 0$
Actiniidae	<i>Teulia crassicornis</i>	P	$\leq 0.1 \pm 0$
Sagartidae	<i>Corynactis californica</i>	P	$\leq 0.1 \pm 0$
Antipathidae	<i>Antipathes atlantica</i>	C	* $3.7 \pm 0.5$
Antipathidae	<i>Antipathes rhipidion</i>	C	$0.2 \pm 0.1$
(Hydrozoa)			
Bougainvilleidae	<i>Hydractinia milleri</i>	P	* $9.8 \pm 2.1$
Tubulariidae	<i>Tubularia crocea</i>	P	$\leq 0.1 \pm 0$
Eudendriidae	<i>Eudendrium californicum</i>	P	$0.2 \pm 0$
Hydridae	<i>Pelmatohydra pseudoelegactis</i>	f.w.	$\leq 0.1 \pm 0$
Hydridae	<i>Chlorohydra viridissima</i>	f.w.	$\leq 0.1 \pm 0$
Sertulariidae	<i>Sertularia turgida</i>	P	* $11.2 \pm 2.9$
Campanulariidae	<i>Clytia bakeri</i>	P	$\leq 0.1 \pm 0$
Plumulariidae	<i>Aglaophenia struthionides</i>	P	$\leq 0.1 \pm 0$
Milleporidae	<i>Millepora alcicornis</i>	C	* $10.3 \pm 2.4$
Milleporidae	<i>Millepora complanata</i>	C	* $8.8 \pm 3.0$
Milleporidae	<i>Millepora squarrosa</i>	C	* $8.3 \pm 4.3$
Stylasteridae	<i>Allopora porphyra</i>	P	* $4.6 \pm 2.2$
Chondrophorae	<i>Velevella velevella</i>	P	$\leq 0.1 \pm 0$



The PGEP synthetase reaction stimulated by salt remains autocatalytic, and thus, difficult to measure; assays monitoring the yield of PG products have proven unreliable for accurate and comparative quantitations of enzymatic activity (Corey *et al.*, 1973; Samuelsson *et al.*, 1975; Miyamoto *et al.*, 1976; Lands and Rome, 1976). Using the spectrophotometric assay with a continuously recording spectrophotometer, however, reliable determinations of the rate of the autocatalytic, salt-stimulated reaction catalyzed by PGEP synthetase in coelenterate extracts were obtained. This maximal rate is directly proportional to the amount of extract added (Table I), and is thus useful for comparative quantitations of enzyme activity.

Previous work from this laboratory has demonstrated that the PGEP synthetases from a variety of marine invertebrates can be activated by hydrogen peroxide; this activation proceeds with immediate elimination of the autocatalytic lag in the PGEP synthetase-catalyzed reaction, and thus makes possible the convenient quantitation of the enzyme with simple first-order kinetics (Morse *et al.*, 1977, 1978). Similar activation (with  $H_2O_2$  in place of NaCl; see Table I) makes possible the direct and convenient quantitation of the enzyme from a wide variety of marine coelenterates.

That  $H_2O_2$  is apparently generated by the enzyme reaction itself, and is thus responsible for the autocatalytic activation (observed in the absence of added peroxide), is indicated by the finding that the addition of *catalase* (0.1  $\mu\text{g}/\text{ml}$ ) to the reaction-mixture ( $\pm 1 \text{ M NaCl}$ ) completely eliminates both autocatalytic activation and all catalytic activity of the enzyme in extracts of the coelenterates *Pleuraura*, *Pseudopleuraura*, *Antipathes*, *Millepora*, and *Allopora*. Similar evidence has been found for the enzyme from marine molluscs and echinoderms (Morse *et al.*, 1977, and unpublished observations), and thus appears to reflect a general property of the reaction-mechanism of this enzyme from many invertebrate species. A role for copper at the active site of these enzymes has been postulated in the generation of  $H_2O_2$  (Morse *et al.*, 1978), and is, in part, supported by the sensitivity of these enzymes to the copper-chelator, DDTC (Table I; Morse *et al.*, 1977, 1978). In these respects, as well as in the relatively low sensitivities to the anti-inflammatory drugs which are potent inhibitors of the mammalian enzymes, the properties of the PGEP synthetases from the marine invertebrates differ from those of the enzymes from mammalian sources (see also Corey *et al.*, 1973).

From a practical point of view, there are several advantages which use of the peroxide-stimulated reaction affords over measurement of the salt-stimulated reaction. Accurate measurements of the maximal autocatalytic rate of the salt-stimulated reaction require sophisticated electronic equipment for continuous monitoring and recording of the spectrophotometric assay. In contrast, the initial rate of the (first-order) peroxide-stimulated reaction can be measured readily in the field, with a simple spectrophotometer (or colorimeter) and stopwatch.  $H_2O_2$  itself is unstable in dilute solution, and in concentrated form (or as the solid, *e.g.*, sodium peroxide) is both caustic and potentially explosive, and thus subject to internationally regulated precautions in transport. However, the peroxide-stimulated reaction can be further adapted to use in the field by replacement of  $H_2O_2$  with a stable enzymatic  $H_2O_2$ -generating system (Table II). When used

with miniaturized and highly portable spectrophotometric equipment, this procedure makes convenient and reliable assays under field conditions possible, allowing comparisons of the specific activities of PGEP synthetase from live, freshly collected specimens of a variety of coelenterates from the Caribbean and eastern Pacific.

Use of these procedures has confirmed the identification of *Plexaura homomalla* as a species exceptionally rich in PGEP synthetase (Table III; Corey *et al.*, 1973; Weinheimer and Spraggins, 1969; Bayer and Weinheimer, 1974). In addition, this study has identified several related plexaurids, as well as certain other Gorgonacea, "Hydrocorallia", Antipatharia, Scleractinia, and Hydroida as species warranting further investigation as sources of potentially great PGRC biosynthetic activity. Although little systematic pattern is discernible in the distribution of the high levels of PGEP synthetase observed, it may be significant that all of the hydrocoral species tested (three *Millepora*, one *Stylasterina*) were found to have exceptionally high levels of this enzyme. Since the total productivity of Pacific and Atlantic species (particularly of the tropical hydrocorals) thus identified far exceeds the relatively low productivity of the Caribbean gorgonian *P. homomalla* (Hinman, 1974; Jordan, *et al.*, 1978), these findings may serve to relieve and diversify pressure for exploitation upon this latter and potentially threatened species.

Marine coelenterates are the phylogenetically simplest organisms in which significant levels of PGEP synthetase thus far have been found. Such activity was not detected in several species each of freshwater Protozoa and marine Porifera. Specific activities of enzyme in the most active coelenterate extracts (Table III) exceed those found in mammalian reproductive tissues, although they are about 50% lower than the highest values found in the eggs of abalone, *Haliotis* spp., and the urchins, *Strongylocentrotus* and *Lytechinus* spp. (Morse *et al.*, 1977, and unpublished observations). Although data implicate this enzyme in the control of reproductive processes in both abalones (Morse *et al.*, 1977, 1978) and urchins (Jensen and Morse, unpublished observations), there is as yet no information regarding the physiological functions of the especially active PGEP synthetases of the marine coelenterates. Similarly, the final (PGRC) products of the enzyme from these sources, with the exception of those from *P. homomalla*, remain to be identified.

The apparent distribution of PGEP synthetase activity found in the marine coelenterates (Table III) might reflect some pattern of seasonal variation, perhaps in reproductive or other specialized functions and/or tissues. However, no such seasonal variation has been detected in samples of five of the Pacific species (*Allopora*, *Sertularia*, *Lophogorgia*, *Muricca*, and *Tealia*) collected and assayed at intervals throughout the year. It is possible, then, that the high levels of PGEP synthetase characteristic of certain species may reflect a role in some fundamental process such as the regulation of ion- and water-transport, as originally suggested by Christ and Van Dorp (1972).

Alternatively, the potent PGRCs in these species might play some role in defense against predation or parasitism, or in specialized aggressive or prey-securing functions. The effectiveness of the PGRCs from molested and damaged colonies of *P. homomalla* in causing severe irritation and other symptoms of intoxication in human collectors has been documented previously (Brooks and

White, 1974). For a discussion of the many physiological functions in which prostaglandins and PGRCs have been implicated, the reader is referred to the recent comprehensive reviews edited by Karim (1975, 1976).

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#### SUMMARY

A convenient and reliable assay is described for PGEP synthetase, the rate-limiting enzyme determining the total capacity for biosynthesis of prostaglandin-related compounds. Results of such assays, performed with fresh specimens under both field and laboratory conditions, newly identify several marine coelenterate species as potentially important resources of PGRCs for research and possible development. Properties of the typical marine coelenterate PGEP synthetase, and the reaction which this enzyme catalyzes, have been further characterized.

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